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Tricyclazole Induced Alterations in Certain Biomarker Enzymes of an Indian Paddy-field Fish, *Channa punctatus* (Bloch)

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ABSTRACT

Tricyclazole, a systemic fungicide is recommended to treat of diseases in irrigated rice. *Channa punctatus* (Bloch) is a freshwater fish also found in paddy fields. This maiden study was designed to evaluate acute toxicity of tricyclazole and its responses of certain biomarker enzymes in *Channa punctatus*. By regression analysis method, 24, 48, 72 and 96hr-LC_{50} dose of tricyclazole was calculated 54.30, 36.76, 32.63 and 25.06 mg l^{-1} respectively. The range of LC_{50} dose indicates highly toxic nature of tricyclaozole. Fish were then exposed to 0.25 and 1.25 mg l^{-1} sublethal dose of tricyclazole for short term (24, 48 and 96 hours) and long term (15, 30 and 45 days) exposure and the alterations of enzyme activities were determined. Alkaline phosphatase activities exposed to 0.25 mg l^{-1} were increased insignificantly ($p>0.05$) after 24 hours but increased significantly ($p<0.05$) to 0.25 and 1.25 mg l^{-1} tricyclazole at all other treatments. Alanine transaminase and aspartate transaminase increased significantly ($p<0.05$) in response to tricyclazole during both exposures. These findings may be used in the assessment of the potential risk of tricyclazole on food chain and aquatic ecosystems.

Key words: Biomarker enzymes, *Channa punctatus*, Toxicity, Tricyclazole.

INTRODUCTION

Arrah is an agricultural area of Bihar dominating paddy culture especially aromatic rice. *Sonachur, Karibank, Basmati, Badshahbhog* and *Kanakjeera* are the scented rice varieties of this area. During last few years the export of Basmati rice to USA and UK has been adversely affected by the presence of residues of tricyclazole in rice.

Rice blast disease is a destructive and wide spread diseases caused by *Pyricularia oryzae* (Naik *et al.* 2012). Tricyclazole is recommended for to treat diseases in irrigated rice and other plants (Anwar and Bhat 2005; Mazumder *et al.* 2016). Studies indicated that tricyclazole has a high risk of environmental contamination (Padovani *et al.* 2006; Sancho *et al.* 2009). The toxicity test gives information about toxicity of a substance in test animals (USEPA 2002).

Channa punctatus (Bloch), a freshwater Indian air breathing fish also found in paddy fields. Studies on biomarker enzymes help in understanding the relationship of biochemical parameters to the habitat and adaptability of fish to the environment (Sancho *et al.* 2009). However, the toxic effect of tricyclazole on this fish is lacking in this context.

Therefore, the work was designed to evaluate LC_{50} dose of tricyclazole and alterations in certain hepatic enzymes in *Channa punctatus*. The study will help to evaluate variation in health of the fish due to toxicity of tricyclazole, suitability of environmental conditions and relative sensitivity of fish to fungicide.

MATERIALS AND METHODS

Channa punctatus (measuring 25-30 g and 12-14 cm) were collected from local fishermen of Arrah and adjacent localities during breeding season of 2017-2019. The fishes were washed properly with dilute $KMnO_4$ and transferred to aquaria. The fishes were fed with fish food available in the local market. The experiments were conducted every time in between 11 AM to 1 PM. The physico-chemical features of experimental water were determined following standard method (APHA, 2005).

Tricyclazole (75% EC; mol formula: $C_9H_7N_3S$; mol mass: 189.24 g mol^{-1}), a fungicide manufactured by Bayer Crop Science Ltd., Gujarat, India was selected for this work. In each selected concentration of Tricyclazole, 10 fishes from the acclimatized fish stock were kept. The first set of experiment was conducted for 24hours, second for 48hours, third for 72hours and fourth for 96hours respectively. The experiments were monitored and numbers of fishes died during the experiments in each concentration was recorded. With the help of the records of dead fishes, 96h-LC_{50} doses were determined by Graphical method taking mortality, percent mortality and probit mortality using regression equations.

The blood samples were collected by heart puncture method. Blood were drawn and transferred into sterilized test tubes containing EDTA and maintained for coagulation.

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After coagulation, the uncoagulated and coagulated part were separated and centrifuged for 10 minutes at 3000 rpm. The supernatant was used to analyze alkaline phosphatase, aspartate transaminase and alanine transaminase following the methods of Balasubaramanian (1983), Splittstoesser (1976) and Bergmeyer and Bernt (1974) respectively.

Statistical analysis was performed with the use of Graph Pad Prism 5.

RESULTS AND DISCUSSION

The temperature 26.0±2.0°C, pH: 7.14±0.08, dissolved oxygen: 6.4±0.4mg l^{-1}, total alkalinity: 56.0±4.5mg l^{-1}, hardness: 150.6±5.2mg l^{-1} and chloride: 16.7±0.2mg l^{-1} of experimental water was recorded to which fish were exposed.

LC_{50} dose

The data on determination of LC_{50} have been depicted in Table 1. The lethal range was determined from 0.01, 0.1, 1.0, 10.0 and 100.0 mg l^{-1} of tricyclazole following the method of Reish and Oshida (1987). No mortality was observed in 0.01 to 10.0 mg l^{-1} of tricyclazole. On the other hand, 100% mortalities were found in 100.0 mg l^{-1} of tricyclazole.

The dose of tricyclazole was converted into log dose and mortality as percent mortality as probit mortality. By this method, 24, 48, 72 and 96hr-LC_{50} of tricyclazole was calculated 54.30, 36.76, 32.63 and 25.06 mg l^{-1} respectively (Table 1 and Fig 1-5). The range of 5-50 mg l^{-1} of LC_{50} dose indicates highly toxic nature of a toxicant (Loomis and Hayes 1996). The present observation indicates that tricyclazole is a highly toxic fungicide.

The LC_{50} values were determined for *Lepomis macrochirus* (2460 µg l^{-1}) and *Oncorhynchus mykiss* (1801 µg l^{-1}) (USEPA 2013) for tricyclazole. The 96hr-LC_{50} dose of tricyclazole of 15.00 mg l^{-1} to *Channa leucopunctatus* was reported by Shilpa *et al.* (2009). They reported that this fungicide was used at 1000 mg l^{-1} in the fields. Pan and Liang (1993) determined LC_{50} of 19425 µg l^{-1} of this fungicide in *Rana limnocharis*.

From this account, it may be inferred that the differences in LC_{50} values influenced by the species, age, weight and habitat of the fish. After determining the value of 96hr-LC_{50} dose of tricyclazole of 25.0 mg l^{-1}, a concentration of 0.25 and 1.25 mg l^{-1} of tricyclazole was selected for this study.

From this work, range of safety level of tricyclazole was calculated from 0.2576x10^{-4} to 1.686 mg l^{-1} in *Channa punctatus*. The range indicates that it is difficult to decide the acceptable safe concentration of tricyclazole in *Channa punctatus*. Moreover, 0.2576 x 10^{-5} to 0.1686 mg l^{-1} and 0.2576 x 10^{-6} to 0.01686 mg l^{-1} of tricyclazole allow a safe level for rat and man respectively (https://en.wikipedia.org/wiki/Toxicity). Large variations in safe levels determined by various methods have resulted in controversy over its acceptability (Pandey *et al.* 2005).

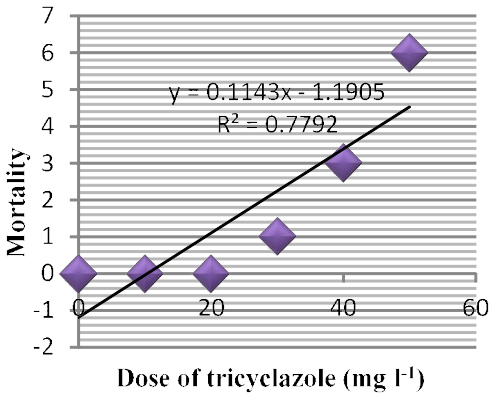

Fig 1: 24hr-LC_{50} dose of tricyclazole in *Channa punctatus.*

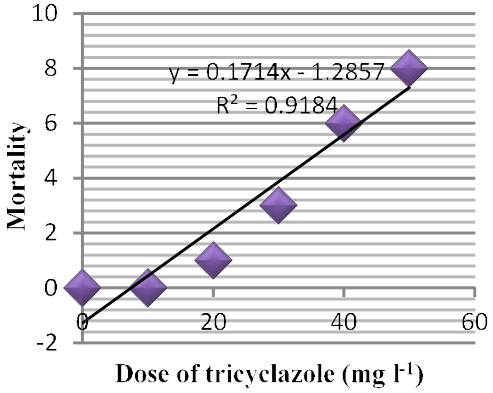

Fig 2: 48hr-LC_{50} dose of tricyclazole in *Channa punctatus.*

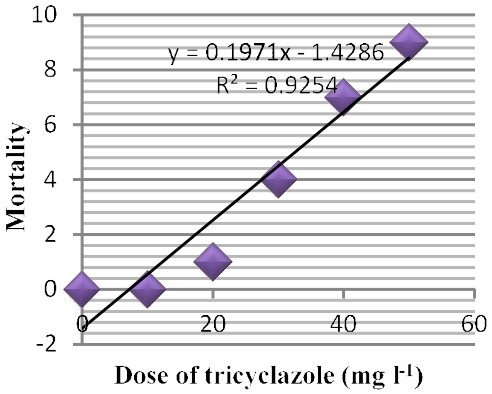

Fig 3: 72hr-LC_{50} dose of tricyclazole in *Channa punctatus.*

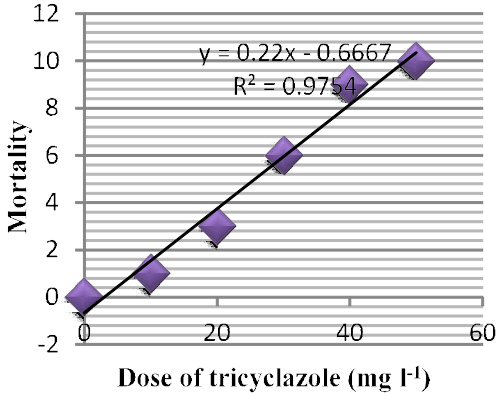

Fig 4: 96hr-LC_{50} dose of tricyclazole in *Channa punctatus.*

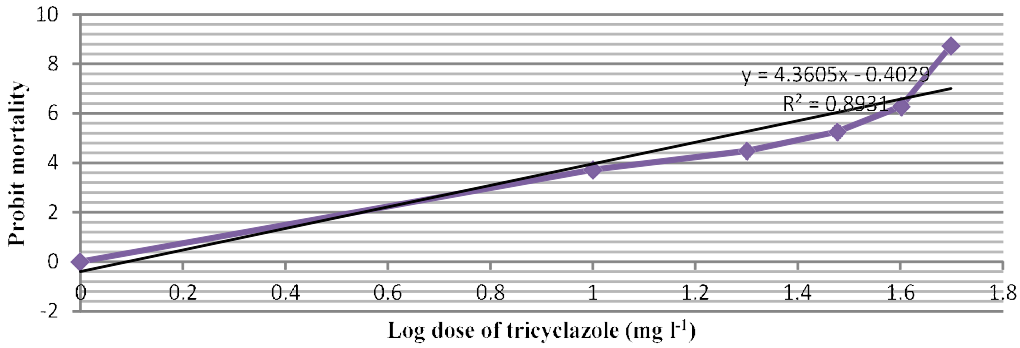

Fig 5: 96hr-LC_{50} dose of tricyclazole in *Channa punctatus.*

Table 1: Statistical relationship between dose of tricyclazole (mg l^{-1}) and mortality of *Channa punctatus.*

Exposure period (hours)	Regression equation y=bx+a (% mortality)	Lethal Concent--ration (mg l^{-1})		Toxicity Factor	t value (df=5)	95% Confidence limit Lower	Higher
24	y=1.143x – 11.90	LC_{10}	19.21			1.84	159.03
		LC_{50}	54.30	1.000	3.774(p<0.05)	18.29	293.03
		LC_{90}	89.38			52.17	460.54
48	y =1.714x – 12.86	LC_{10}	13.37			0.57	44.12
		LC_{50}	36.76	1.477	6.695(p<0.01)	17.07	83.92
		LC_{90}	60.15			33.57	123.72
72	y =1.971x – 14.29	LC_{10}	12.33			0.27	40.04
		LC_{50}	32.63	1.664	6.971(p<0.01)	14.83	73.54
		LC_{90}	52.94			29.38	107.04
96	y =2.200x – 6.667	LC_{10}	7.58			0.74	18.27
		LC_{50}	25.06	2.108	14.1(p<0.001)	15.64	41.60
		LC_{90}	43.94			30.54	64.92

Biomarker enzymes

The alkaline phosphatase, aspartate transaminase and alanine transaminase in controlled *Channa punctatus* was 42.15±1.25, 35.57±1.56 and 13.25±1.25 iu l^{-1} were following normal range (Tables 2, 3 and 4). The amount of alkaline phosphatase (69.05±13.04 iu l^{-1}), aspartate transaminase (265.60±56.55 iu l^{-1}) and alanine transaminase (5.65±1.18 iu l^{-1}) in *Acipenser stellatus* was observed by Shahsavani *et al.* (2010). The observed values were less than the values of alkaline phosphatase and aspartate transaminase but more than alanine transaminase of *Acipenser stellatus.* The difference may be due to the variations in the physiological conditions and habitat of fishes.

The tricyclazole intoxicated *Channa punctatus* showed a significant increase from 45.601.54 to 56.094.14 iu l^{-1} in alkaline phosphatase. Analytically, the alkaline phosphatase activities of fish exposed to 0.25 mg l^{-1} increased significantly after 48hours, 96hours and chronic exposure. But, activities of this enzyme increased significantly in fish exposed to 1.25 mg l^{-1} tricyclazole at all sampling intervals (Table 2). It was analyzed that duration of exposure has more significant effect compared to the dose of tricyclazole for alkaline phosphatase in this fish. Alkaline phosphatase plays the major role in phosphate metabolism and prevents the membrane from being damaged (Dirrieu and Tran-Minh 2002). The increased activity of alkaline phosphatase in fish is linked to the increased catabolic tissue breakdown in melanomacrophage centers (Agius and Coushman 1986). Earlier, Agarwal and Sastry (1979) have recorded significant increase in alkaline phosphatase in *Channa punctatus* after short term exposure of mercuric chloride. Observations of Edori *et al.* (2013) with long term sublethal concentrations of paraquat in *Clarias gariepinus* also showed that alkaline phosphatase increased significantly. These earlier findings support the observations of present work.

Aspartate transaminase and alanine transaminase in tricyclazole exposed fish showed significant increase from 51.328.14 to 58.088.78 iu l^{-1} and 17.90.69 to 20.560.51 iu l^{-1} respectively. Analytically, these enzymes in fish exposed to 0.25 and 1.25mg l^{-1} tricyclazole increased significantly after 24hours, 48hours, 96hours and chronic exposure (Table 3 and 4). It was analyzed that dose of exposure has more significant effect compared to the duration of exposure of tricyclazole for these enzymes in this fish. Increase in aspartate transaminase and alanine transaminase has also been reported in *Oreochromis mykiss* and *Channa punctatus,* exposed to organophosphates, diazinon and monocrotophos respectively (Qayoom *et al.* 2019; Banaee *et al.* 2011; Agrahari *et al.* 2006). Observations of Edori *et al.* (2013) with long term sublethal concentrations of paraquat in *Clarias gariepinus* showed that aspartate transaminase increased significantly while alanine transaminase either

Table 2: Variations in serum alkaline phosphatase (iu l^{-1}) of *Channa punctatus* after acute and chronic exposure of two sub-lethal dose of tricyclazole.

Dose (mg l^{-1})	Range	Controlled value	Duration of exposure a=24 hours b=15 days	a=48 hours b=30 days	a=96 hours b=45 days	F value
0.25			43.74±0.54[a] (+3.77%)NS	46.29±0.40[a] (+9.82%)*	49.48±0.33 [a] (+17.39%)**	25.85** and 6.69*
			45.76±0.96[b]	49.74±0.58[b]	55.96±0.43[b]	(at 0.5=5.1,0.1=10.9,
	30.0 -90.0	42.15±1.25	(+8.56%)*	(+18.01%)**	(+32.76%)***	0.01=27.0 for
1.25			44.93±0.94[a] (+6.60%)*	53.67±0.36[a] (+27.33%)***	60.46±0.55[a] (+43.44%)***	n_1=2 and n_2=6)
			47.96±0.45[b]	52.72±0.61[b]	58.46±0.35[b]	(at 0.5=4.8,0.1=9.8,
			(+13.78%)**	(+22.70%)**	(+38.70%)***	0.01=23.7 for n_1=3
Average			45.60±1.54 (+8.19%)*	50.61±2.88 (+20.07%)**	56.09±4.14 (+33.07%)***	and n_2=6)

(NS= Not Significant, *= Significant, **= Moderately Significant, ***= Highly Significant).

Table 3: Variations in serum aspartate transaminase (iu l^{-1}) of *Channa punctatus* after acute and chronic exposure of two sub lethal dose of tricyclazole.

Dose (mg l^{-1})	Range	Controlled value	Duration of exposure a=24 hours b=15 days	a=48 hours b=30 days	a=96 hours b=45 days	F value
0.25			43.76±0.48[a] (+23.02%)**	45.87±0.86[a] (+28.96%)***	49.86±0.29[a] (+40.17%)***	117.8*** and 1085***
			42.67±0.69[b]	45.17±0.89 [b]	48.96±1.29[b]	(at 0.5=5.1,0.1=10.9,
	30.00 -320.00	35.57±1.56	(+19.96%)**	(+26.99%)**	(+37.64%)***	0.01=27.0 for
1.25			60.39±0.49[a] (+69.78%)***	63.47±0.85[a] (+78.44%)***	68.64±0.53[a] (+92.9%)***	n_1=2 and n_2=6)
			58.47±0.45[b]	60.92±0.86[b]	64.86±0.96[b]	(at 0.5=4.8,0.1=9.8,
			(+64.38%)***	(+71.27%)***	(+82.34%)***	0.01=23.7 for
Average			51.32±8.14 (+44.28%)***	53.86±8.39 (+51.42%)***	58.08±8.78 (+63.28%)***	n_1=3 and n_2=6)

(**= Moderately Significant, ***= Highly Significant).

Table 4: Variations in serum alanine transaminase (iu l^{-1}) of *Channa punctatus* after acute and chronic exposure of two sub lethal dose of tricyclazole.

Dose (mg l^{-1})	Range	Controlled value	Duration of exposure a=24 hours b=15 days	a=48 hours b=30 days	a=96 hours b=45 days	F value
0.25			17.10±0.23[a] (+24.36%)**	18.2±0.20[a] (+32.36%)***	21.2±0.31[a] (+54.58%)***	7.35* and 12.30**
			18.30±0.24[b]	19.1±0.31[b]	20.8±0.34[b]	(at 0.5=5.1,0.1=10.9,
	5.00 -90.00	13.25±1.25	(+33.09%)***	(+9.82%)**	(+51.27%)***	0.01=27.0 for
1.25			18.83±0.22[a] (+20.79%)**	19.67±0.34[a] (+43.05%)***	20.44±0.26[a] (+48.65%)***	n_1=2 and n_2=6)
			17.37±0.20[b]	18.71±0.28[b]	19.82±0.62[b]	(at 0.5=4.8,0.1=9.8,
			(+26.33%)**	(+36.07%)***	(+44.14%)***	0.01=23.7 for
Average			17.9±0.69 (+30.18%)***	18.92±0.54 (+32.36%)***	20.56±0.51 (+49.53%)***	n_1=3 and n_2=6)

(**= Moderately Significant, ***= Highly Significant).

decreased or increased significantly. Haider and Rauf (2014) also reported that aspartate transaminase and alanine transaminase increased significantly in *Cirrhinus mrigala* exposed to diazinon compared to the controlled fish.

The increased levels of aspartate transaminase and alanine transaminase in *Channa punctatus* indicate that long-term exposure to tricyclazole caused tissue damage in fish. The reactive oxygen species produced during tricyclazole and other toxicants metabolism in fish lead to increase in the permeability of gills, hepatocytes, renal cells and cardiac cells resulting in the leakage of aspartate transaminase and alanine transaminase (Srivastava *et al.* 2004).

The ratio of alanine transaminase: aspartate transaminase ($\frac{13.25}{35.57}$ = 0.38) in tricyclazole exposed fish to be less than one indicates manifestation of pathological processes (Table 3 and 4). An increase in transamination occurs due to amino acid input into the Krebs' cycle in order to cope with the energy crisis during pesticide/tricyclazole based stress (Adams *et al.* 1996).

CONCLUSION

Therefore, it may be inferred that the activities of alkaline phosphatase, aspartate transaminase and alanine transaminase increased significantly in serum of tricyclazole exposed *Channa punctatus*. In this context, these enzymes are rightly considered as the pathological biomarker enzymes. Therefore, the entry of such fungicide to natural water bodies should be stopped besides ensuring their judicious application around the catchment area.

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